combined with functional modulation by membrane lipid and water-vestibules. Here, we used electron paramagnetic resonance (EPR) spectroscopy to delineate protein motions underlying GLIC gating in a membrane environment, and report the interface conformation in the closed and the desensitized states. Extensive intra-subunit interactions were observed in the closed state which are weakened upon desensitization, and replaced by newer inter-subunit contacts. Gating involves major rearrangements of the interfacial loops accompanied by reorganization of the protein-lipid-water interface. These structural changes may serve as targets for modulation of gating by lipids, alcohols and amphipathic-drug molecules.

3245-Plat

Calcium-Dependent Gating in MthK K^+ Channels Occurs at the Selectivity Filter

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The primary activation gate in voltage-gated Kv channels is formed by a helix bundle crossing at the intracellular pore entrance. Among the evidence for this model is that, in the presence of intracellular high-affinity quaternary ammonium (QA) blockers, Kv channel activation is followed by a slow block that is indicative of gated access of the blocker to a binding site inside the pore. For several types of ligand-gated channels such as CNG and large-conductance Ca^{2+}-activated K^+ (BK) channels, however, the channels' selectivity filter has been proposed to act as the conduction gate, in which case gated blocker access is not expected. We investigated the location of the ligand-controlled gate in the calcium-activated MthK channel, a prokaryotic BK channel homologue lacking voltage-sensor domains, using an assay for closed-state channel block. In order to achieve fast MthK activation, we employed a Tl^+ flux assay using a stopped-flow spectrofluorometer. Purified MthK channels were reconstituted into liposomes loaded with the fluorophore ANTS and channel activity was estimated from the rate of ANTS quenching due to Tl^+ influx through the channels. 10 mM Ca^{2+} resulted in an activation rate comparable to the 2 ms mixing dead time. Using a sequential mixing protocol to apply the high-affinity QA blockers tetrapentylammonium or bbTBA prior to channel activation, we found that these molecules were able to fully block closed channels, indicating that the QA blockers can reach their binding site in closed channels. Given that the QA binding site is in the aqueous cavity below the selectivity filter, these results suggest that there is a calcium-dependent gate at the selectivity filter in MthK.

3246-Plat

Closed State Coupled C-Type Inactivation in BK Channels

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C-type inactivation of voltage-gated ion channels commonly occurs from the open state (open-sate inactivation, OSI) at strongly positive membrane potentials or from pre-open closed states (closed-state inactivation, CSI) at modestly depolarized membrane potentials. Slow recovery from inactivation requires hyperpolarized membrane potentials. C-type inactivation is known to result from structural rearrangement or collapse at selectivity filter. BK channels are known to be different from most voltage-gated K^+ channels due to the absence of a classic intracellular cross-bundle activation gate, prompting suggestions of an opening mechanism due to rearangements of the selectivity filter. We studied C-inactivation in BK channels to explore the hypothesis that BK channel's activation and C-inactivation may have closely related gating mechanisms. The BK channel doesn't have C-inactivation under physiological conditions because of its high affinity to external K^+. We observed prominent slow C-inactivation and recovery upon providing two stimulating factors of C-inactivation: (1) mutation Y294E/K/Q/S or Y279F, whose equivalent in Shaker channels(T449E/K/D/Q/S or W434F) caused greatly increased rates of C-inactivation or constitutive inactivation; (2) a very low concentration of extracellular K^+ by replacement of K^+ with $NMDG^+$. However, BK channels showed a distinct state-dependency of C-inactivation which occurs at negative membrane potentials or decreased $[Ca^{2+}]_i$ that cause channels to close, whereas recovery from inactivation requires positive membrane potentials or elevated $[Ca^{2+}]_i$ that promote channel opening. Constitutively open mutatations prevented BK channel from C-inactivation, suggesting the absence of an open- inactivated state. We conclud that channel closing is a prerequisite although not an equivalent to the C-inactivation in the BK channels, indicating a strong coupling between these two processes. Because C-inactivation can involve multiple conformational changes at selectivity filter, the BK channel closing which can recover (open) relatively quickly might represent an early conformational stage of C-inactivation.

3247-Plat

TPC1 is a Proton Permeable Channel that can be Independently Activated by Cytosolic Calcium or NAADP

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Nicotinic acid adenine dinucleotide phosphate (NAADP) potently releases Ca^{2+} from acidic intracellular lysosomal Ca^{2+}-stores. There is evidence that two-pore channels (TPCs), a family of ion-channels localised to the acidic stores, are involved in NAADP-mediated Ca^{2+}-release. We have shown that human TPC type-2 (TPC2) possesses certain key biophysical properties that suggest it may function as a lysosomal NAADP-sensitive Ca^{2+}-release channel [1]. We now investigate whether two-pore channel type-1 (TPC1) exhibits similar functional properties by reconstituting purified human TPC1 into artificial membranes under voltage-clamp conditions. Similar to TPC2, we find that TPC1 is impermeable to Cl^- but is poorly selective among cations with conductance decreasing in the order $K^+ > Na^+ > Ca^{2+}$. We also find that, unlike TPC2, TPC1 is highly permeable to protons; under bi-ionic conditions, relative permeability decreases in the order $H^+ >> K^+ > Na^+ > Ca^{2+}$. Significantly, we show that the regulation of TPC1 gating is very different to that of TPC2. We previously demonstrated that TPC2 activity is not regulated by cytosolic Ca^{2+} [2] but here find that TPC1 can be activated by nanomolar concentrations of NAADP alone or by cytosolic Ca^{2+} alone. This fundamental difference in channel regulation will enable TPC1 to be activated independently from TPC2 allowing the endo-lysosomal system to respond to physiological needs by integrating a variety of cellular signals. We suggest that in the endolysosomal and lysosomal systems, TPC2 can effectively function as a NAADP-activated Ca^{2+}-release channel. In contrast, TPC1 can leak protons from these acidic Ca^{2+}-stores and although TPC1 is capable of providing some degree of Ca^{2+}-release, this is heavily dependent on K^+ and Na^+ gradients across the lysosomal membrane, on the lysosomal membrane potential, the luminal $[Ca^{2+}]$ and the luminal pH.

[1].Pitt *et al.*,(2010)JBC;**285**:35039-46.
[2].Pitt *et al.,(*2011)Biophys.J;**100**:433a.

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3248-Plat

SKA-111, a Positive KCa Channel Gating Modulator with Selectivity for KCa3.1

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Intermediate and small-conductance Ca^{2+} activated potassium (KCa) channels play an important role in regulating membrane excitability and Ca^{2+} signaling. Pharmacological activation of these voltage-independent channels has therefore been suggested for the treatment of various diseases. While KCa2 activators can reduce neuronal excitability in CNS disorders, KCa3.1 activators are being discussed as endothelial targeted antihypertensives. KCa channels are gated by Ca^{2+} binding to a constitutively associated calmodulin at the C-terminus. Recent work (Zheng M. 2012 Nat Commun 3:1021) demonstrated that the benzimidazolone 1-EBIO binds at the interface of the CAM N-lobe and the calmodulin binding domain (CAMBD) of KCa2.2. Mutations of A477V/L480M and A477I in KCa2.2 were shown to increase and decrease potency of 1-EBIO, respectively. Here we report that the same mutations (A625V/L628M and A625I) in KCa2.3 also alter the potency of KCa2/3 activators of the benzothiazole class such as our recently developed SKA-31, a pharmacological tool compound that has been widely used to study the role of KCa2 and KCa3.1 channels. However, like 1-EBIO and NS309, SKA-31 suffers from a lack of selectivity between KCa3.1 and KCa2 channels making it difficult to interpret in vivo observations made with this compound. Via a structure activity relationship (SAR) study we have optimized the pharmacophore of SKA-31 and have now identified SKA-111 (5-methylnaphtho[1,2-d]thiazol-2-amine), which displays 160-fold selectivity for KCa3.1 (EC50 = 118 ± 29 nM) over KCa2.3 (EC50 = 11.2 ± 4 μM). SKA-111 constitutes a novel pharmacological tool to study the role of KCa3.1 in blood pressure regulation. SKA-111 selectively potentiates native KCa3.1 currents in porcine carotid endothelium and lowers blood pressure in mice. SKA-111 should further be useful to help understand what structural elements confer selectivity between KCa3.1 and KCa2 activation to the benzothiazole pharmacophore.